Aquaculture Products
Supplementary Sampling and Laboratory Testing Instructions
(Issue 1.0 – 5-September-2019)

Seafood Processing Standard (SPS)
(Issue 5.0 – 01-February-2019)

Use in conjunction with ANNEX 4 – Sampling and Testing Verification Requirement

1.0 Primary Product Form

As referred to in the SPS Standard – “Primary Product Form” examples are:

- fresh
- raw ready-to-eat (e.g. sashimi)
- raw frozen
- raw breaded
- cooked breaded
- cooked dumpling
- smoked - cold
- smoked - hot
- pickled
- dried
- canned
- salted
- marinated

Raw frozen product forms, for the purpose of this definition, include all raw IQF or block frozen products expected to have the same hazards (e.g. microbiological). Examples include: deveined, peeled and deheaded, whole, deheaded, butterflied tail on.

Example 1 – Primary Product Form:
A plant produces L. vannamei of the following forms: cooked, breaded, raw ready to eat, marinated, and 3 forms of peeled and/or deveined for a total of 7 products:

- The number of primary product forms to be sampled = 5, (Cooked, breaded, raw ready-to-eat, marinated, and raw).
- When sampling, primary product forms are based on a per species basis. So if the plant above was also producing raw P. monodon in frozen block and IQF, that would be 5 primary product forms for L. vannamei and 1 for P. monodon.
2.0 Sampling Instructions

2.1 Number of Samples

a. The number of samples to collect per species is based on annual production volumes of each BAP certifiable species produced at the processing facility according to Table 1.

<table>
<thead>
<tr>
<th>Volume (MT)</th>
<th>Number of Samples per Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-1,000</td>
<td>4</td>
</tr>
<tr>
<td>1,001-3,000</td>
<td>8</td>
</tr>
<tr>
<td>&gt;3000</td>
<td>12</td>
</tr>
</tbody>
</table>

**Example 2 – Number of samples per species:**

A processing facility previous year’s production record states the following:

- **L. vannamei** 3,500 MT
- **P. monodon** 1,200 MT
- **O. niloticus** 800 MT

On the day of sampling, the following product forms are observed in inventory and storage:

- White shrimp (**L. vannamei**) – raw frozen IQF, raw frozen block, breaded frozen, cooked frozen
- Black tiger shrimp (**P. monodon**) – raw frozen IQF, raw frozen block
- Nile tilapia (**O. niloticus**) – raw frozen fillet, breaded frozen fillet

Total number of samples to take = 24 as follows:

- **L. vannamei** = 12 samples randomly selected between raw, breaded and cooked primary product forms. In this instance, raw frozen IQF and raw frozen block are considered a single primary product form.
- **P. monodon** = 8 samples randomly collected between raw production lots.
- **O. niloticus** = 4 samples randomly selected between fillet and breaded production lots.

An acceptable sample collection for the above example is shown below:

```
<table>
<thead>
<tr>
<th>L. vannamei</th>
<th># Samples</th>
<th>P. monodon</th>
<th># Samples</th>
<th>O. niloticus</th>
<th># Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw frozen IQF</td>
<td>2</td>
<td>Raw frozen IQF</td>
<td>4</td>
<td>Raw frozen fillet</td>
<td>2</td>
</tr>
<tr>
<td>Raw frozen block</td>
<td>3</td>
<td>Raw frozen block</td>
<td>4</td>
<td>Breaded frozen</td>
<td>2</td>
</tr>
<tr>
<td>Breaded frozen</td>
<td>4</td>
<td>Total</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cooked frozen</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>12</strong></td>
<td><strong>Total</strong></td>
<td><strong>8</strong></td>
<td><strong>Total</strong></td>
<td><strong>4</strong></td>
</tr>
</tbody>
</table>
```

Total primary product forms = 3
Primary product forms = 1
Primary product forms = 2
2.2 Sample Collection Requirements

a. Compositing of samples is to be conducted by a qualified third-party laboratory not the sampler.

b. Collect up to twelve samples (750 grams each) as described above from among the primary product forms of finished products that are in the active inventory at the processing plant at the time of the audit.

c. In cases where a processing plant does not have the required number of different production lots in inventory for each species (which may occur in small plants or in plants that produce only fresh products), additional samples will have to be collected from higher-risk or higher-volume production lots to reach the required number of samples.

d. For Seafood Processing Plants that also process wild-caught species, follow these instructions for aquaculture products. Additional samples shall be collected for wild-caught species under separate instructions.

e. Aseptic sampling protocols shall be followed at all times.

f. Sampling shall be conducted in accordance with SPS 5.0 ANNEX 4, sections A4_1.0-3.0 and Table I.

g. Samplers shall organize and obtain equipment necessary to conduct the sampling: thermo-cool boxes, sterile polyethylene bags (clarify firstly with the assigned laboratory concerning their standard procedures, as they may refuse the sample if improper packaging procedures are used), heat-sealing machines (normally available at the plant), and permanent markers (do not attempt to use stickers, as they may not stick properly to sample bags).

h. DO NOT USE black Sharpie markers to identify sample alphanumeric sample codes on sample bags due to the findings that they may contain prohibited dyes utilized in aquaculture.

i. Samplers shall organize the traceability/chain of custody elements related to samples that will be collected, along with the means to document it.

j. Samplers shall inform the lab of the expected date and time for delivery of samples - especially if it is out of normal business hours for the lab, so that they can make arrangement to store the samples accordingly.

k. Samples shall be packaged in an outer package containing only individual samples from a single species.

3.0 Laboratory Testing Instructions

a. Testing laboratories must be accredited to ISO 17025

b. Compositing of samples is allowed given the following conditions:
   i. Compositing is to be conducted by a qualified third-party laboratory not the sampler.
ii. Before compositing is done, samples shall be split so there will be reserve portions of each sample available in case follow-up breakout testing for one or more parameters is required.
   iii. No more than 4 samples can be combined into a single composite.
   iv. No compositing between aquaculture (farmed) products and wild-caught fishery products is allowed.
   v. Compositing across primary product forms is acceptable for drug residue testing only. Mixing primary product forms is not allowed for microbiological tests.

c. Testing shall be in accordance with ANNEX 4 Tables I, II, III.
d. Laboratories shall determine the number of tests based on the number of samples received per species according to Table 2 (below). MT is provided as a reference only for laboratories as they will be unaware of plant volumes.

NOTE: Shellfish shall be tested for microbiological parameters only (Annex 4 Table I). Drug residue testing is not required on shellfish.

Table 2. Residue and microbiological test enumerations based on metric tonnage (MT) and number of samples submitted per species.

<table>
<thead>
<tr>
<th>MT</th>
<th>Number of Samples per Species</th>
<th>Number of Residue Tests per Species</th>
<th>Number of Microbiological Tests per Species</th>
<th>Number of primary product forms Present per Species</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>0-1,000</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>≧ 2</td>
</tr>
<tr>
<td>1,001-3,000</td>
<td>8</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>&gt;3000</td>
<td>12</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

Maximum number of samples in a composite = 4
Maximum number of samples in a composite ≧ 4
Composites may contain mixed primary product forms
Composites must contain the same primary product form - no mixing of primary product forms.

Example 3 – Laboratory testing enumerations for aquaculture species:
The testing laboratory receives 24 individual samples for product testing as indicated in example 2 above:

<table>
<thead>
<tr>
<th>L. vannamei</th>
<th># Samples</th>
<th>P. monodon</th>
<th># Samples</th>
<th>O. niloticus</th>
<th># Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw frozen IQF</td>
<td>2</td>
<td>Raw frozen IQF</td>
<td>4</td>
<td>Raw frozen fillet</td>
<td>2</td>
</tr>
<tr>
<td>Raw frozen block</td>
<td>3</td>
<td>Raw frozen block</td>
<td>4</td>
<td>Breaded frozen</td>
<td>2</td>
</tr>
<tr>
<td>Breaded frozen</td>
<td>4</td>
<td>Total</td>
<td>8</td>
<td>Total</td>
<td>4</td>
</tr>
<tr>
<td>Cooked frozen</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Microbiological and Drug Residue Testing can be determined as follows:

**Microbiological Testing:**

- **L. vannamei:** 12 samples total. Number of microbiological tests required = 3 (Table 2).
  Composite up to 4 individual samples per composite by primary product form. Raw frozen IQF and raw frozen block are considered a single primary product form and can be composited together.
  Examples of acceptable composites:
    - Composite 1: two samples (lots) each of raw frozen IQF and raw frozen block
    - Composite 2: four samples breaded frozen
    - Composite 3: three samples cooked frozen

- **P. monodon:** 8 samples total. Number of microbiological tests required = 2.
  Composite 4 individual samples per composite in any combination. Note that since all samples are raw primary product form they can be composited together.

- **O. niloticus:** 4 samples total. Number of microbiological tests required = 2. Lots obtained are from different primary product forms and cannot be composited.
  Examples of acceptable composites:
    - Composite 1: two lots of raw frozen fillet
    - Composite 2: two lots of breaded frozen

**Drug Residue Testing:**

- **L. vannamei:** 12 samples total. Number of drug residue tests required = 2 (Table 2).
  Composite 4 individual samples per composite in any combination. When possible, laboratories may try to separate raw with cooked samples in compositing; however, mixing across primary product forms is acceptable for drug residue testing only. Note that in this case a maximum of 8 samples could be combined into 2 composites (2 composites containing 4 samples each), leaving 4 samples untested for drug residues.

- **P. monodon:** 8 samples total. Number of drug residue tests required = 2.
  Composite 4 individual samples per composite in any combination.

- **O. niloticus:** 4 samples total. Number of drug residue tests required = 1.
  Composite 4 individual samples into 1 composite.

**4.0 Detections**

4.1 Microbiological Detections
Assessing whether a composited microbiological detection warrants breakout of individual samples is somewhat subjective for MPN serial dilution tests such as *E. coli* and *Staphylococcus aureus*. Table 3 is provided as a guideline:
Table 3. Microbiological criteria rejection limits and BAP Guidelines for determining whether a composited microbiological test detection should be broken out into individual samples for retesting.

<table>
<thead>
<tr>
<th>Microbiological Criteria</th>
<th>Species / Form</th>
<th>Composite Breakout Testing Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Out of 5 subsamples, breakout if 3 or more units exceed 4.0 per gram; 1 or more units exceed 40 bacteria per gram (MPN)</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>Finfish and crustaceans (all forms), and processed/cooked molluscan shellfish</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Out of 5 subsamples, breakout if 1 or more units exceed 165 bacteria per 100g, or if 2 or more units exceed 115 bacteria /100g (MPN)</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>Shell stock, fresh-shucked thawed and frozen shellfish, shellfish frozen on half shell</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>Finfish/crustaceans (all forms)</td>
<td>Reject all samples if equal to or greater than 2,500 bacteria per g (MPN)</td>
</tr>
<tr>
<td>Salmonella sp.</td>
<td>Finfish/crustaceans/molluscan shellfish (all forms)</td>
<td>Reject all samples if presence is detected in 25 grams*</td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>Finfish/crustaceans/molluscan shellfish (cooked and raw, ready to eat products only)</td>
<td>Reject all samples if presence is detected in 25 grams*</td>
</tr>
</tbody>
</table>

* Rejections require immediate notification to the Certification Body and BAP of all potential contaminated lots, to be followed by breakout testing to determine the violative plant production lot(s).

4.2 Drug Residue Detections
A drug residue detection on a composite that is proportionately capable of exceeding limits specified in ANNEX 4 Table III shall be broken out into individual samples (using the reserve portions of the associated sample lots) for confirmatory testing to determine which plant production lot(s) may be adulterated. Table 4 provides BAP guidelines for laboratories to use for breakout testing when a composite detection is obtained.

In the event that breakout testing is conducted, ONLY THE SAMPLES IN THE COMPOSITE PRODUCING THE DETECTION SHALL BE RETESTED (USING THE RESERVE PORTIONS), AND ONLY FOR THE PARAMETER(s) THAT PRODUCED A POSITIVE DETECTION.
Table 4. BAP Guidelines for determining whether a drug residue composite detection should be broken out into individual samples for retesting.

<table>
<thead>
<tr>
<th>Samples per composite</th>
<th>Limit (µg/kg)</th>
<th>Breakout Value (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.3</td>
<td>0.5</td>
</tr>
<tr>
<td>2</td>
<td>0.18</td>
<td>0.30</td>
</tr>
<tr>
<td>3</td>
<td>0.12</td>
<td>0.20</td>
</tr>
<tr>
<td>4</td>
<td>0.09</td>
<td>0.15</td>
</tr>
</tbody>
</table>

Example 4 – Determination of breakout testing for a composite detection:

The testing laboratory conducts drug residue tests per ANNEX 4 on a composite consisting of 4 individual samples. Results indicate a positive detection for Chloramphenicol at any value ≥ 0.09 µg/kg. Per ANNEX 4, the Method LOQ/MRPL for Chloramphenicol is listed as 0.3 µg/kg. Using the guideline in Table 4, any value ≥ 0.09 µg/kg would initiate breakout of the composite, and the 4 samples that comprise the composite should be retested individually for Chloramphenicol.

5.0 Failures

5.1 Microbiological Test Failures
a. Individual samples testing above the Limits in ANNEX 4 Table II are considered a Failed Test Result.
b. Any composite testing above a rejection limit for *Staphylococcus aureus*, *Salmonella* sp., or *Listeria monocytogenes* stated in ANNEX 4 Table II (and Table 3 above), requires immediate notification by the testing laboratory to the overseeing Certification Body and BAP. All potential violative plant production lots shall be identified within the notification. Once the notification has been established, the laboratory shall proceed with confirmatory testing on individual samples comprised in the composite of detection for the Microbiological Criteria detected to determine the violative plant production lot(s).

5.2 Drug Residue Failures
a. A Drug Residue Detection on an individual sample testing above the “Method LOQ/MRPL” value listed in Annex 4 Table III is considered a Failed Test Result.
b. A Drug Residue Detection that is above the Testing Laboratory’s LOQ, but below the “Method LOQ/MRPL” value listed in ANNEX 4 Table III is considered a “Detection”.

For questions regarding these instructions contact BAP Program Integrity programintegrity@bapcertification.org
1-603-242-1575